IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:		§	
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TONY PELED		§	
		§	
Serial No.:	10/764,833	§	
		§	
Filed:	February 09, 2004	§	Group Art Unit: 1633
		§	
For:	EXPANSION OF RENEWABLE STEM CELL POPULATIONS	§	
		§	
		§	Attorney
		§	Docket: **26691**
Examiner: LEAVITT, MARIA GOMEZ, PhD		§	

Commissioner for Patents
POBox 1450
Alexandria VA23313-1450

DECLARATION OF DR. TONY PELED UNDER 37 CFR §1.132

I, Tony Peled, am the Chief scientist and vice president of Gamida-Cell Ltd. at Jerusalem, Israel. My professional specialization is in the field of cell biology, particularly with regard to stem cell culture and therapeutic applications. I have attached a copy of my curriculum vitae with some of the most recent publications.

I am the inventor of the present invention. I have read the present application and the new and amended claims, as well as the Office Action from the Examiner and the accompanying references. In support of the accompanying Response to this Office Action, I set forth below some important experimental data which demonstrates some of the unique characteristics of the claimed invention.

In this Official action, the Examiner has rejected claims 401, 411-412, 414-425, 434, 436-438 and 462 under 35 U.S.C. § 112 1st paragraph, asserting lack of enablement. The results of the hematopoietic stem cell culture described hereinbelow are consistent with the results disclosed in the instant specification, indicating that hematopoietic stem cell cultures maintained under the claimed culture conditions (in the presence of cytokines and nicotinamide) continue to support significant expansion of hematopoietic stem and progenitor cell populations for greater than three weeks

duration of culture.

The instant claims are related to methods for expansion of hematopoietic stem and progenitor cells by culturing sources of hematopoietic stem and/or progenitor cells in the presence of conditions for cell proliferation (e.g. nutrients and cytokines) and inhibitors of CD38 (e.g. nicotinamide), resulting in the expansion of stem and progenitor cell populations, and therapeutic applications of the expanded hematopoietic stem and progenitor cells. Culture conditions resulting in expansion of the desired cell populations are described in detail in the present application.

As an inventor of the present application, I either performed myself or caused to be performed numerous experiments to assess the feasibility of using nicotinamide for expanding hematopoietic stem and progenitor cells in culture for extended periods of time. These experiments are described in greater detail below, along with graphical representation of the results.

Expansion of hematopoietic stem and progenitor cells fraction by culture of hematopoietic cells with nicotinamide for greater than 3 weeks.

Hematopoietic cells maintained in the presence of cytokines and nicotinamide showed continued increased expansion of the total CD34+ cell population, and of the CD34+/CD38- and CD34+/Lin- subpopulations at 3 weeks and at 5 weeks duration of culture. The experiments themselves are described in greater detail below.

Mononuclear cell fraction collection and purification:

Human blood cells were obtained from umbilical cord blood from female patients following full-term, normal delivery (informed consent was obtained). Samples were collected and processed within 12 hours postpartum. Blood was mixed with 3 % Gelatin (Sigma, St. Louis, MO), sedimented for 30 minutes to remove most red blood cells. The leukocyte-rich fraction was harvested and layered on a Ficoll-Hypaque gradient (1.077 gram/ml; Sigma), and centrifuged at 400 *g* for 30 minutes. The mononuclear cell fraction in the interface layer was collected, washed three times and resuspended in phosphate-buffered saline (PBS) solution (Biological Industries) containing 0.5 % bovine serum albumin (BSA, Fraction V; Sigma).

Purification of $CD34^+$ cells from mononuclear cell fractions:

To purify $CD34^+$ mononuclear cells, the fraction was subjected to two cycles of immuno-magnetic separation using the MiniMACS® or Clinimax® CD34 Progenitor Cell Isolation Kit (Miltenyi Biotec, Auburn, CA) as per manufacturer's

recommendations. The purity of the $CD34^+$ population obtained ranged from 95 % to 98 % as was determined by flow cytometry (see below).

To further purify the $CD34^+$ population into $CD34^+38^-$ or the $CD34^+ Lin^-$ sub-fractions, the purified $CD34^+$ cells were further labeled for CD38 (Dako A/S, Glostrup, Denmark) or lineage antigens (BD Biosciences, Erermbodegem, Belgium). The negatively labeled fraction was measured and sorted by a FACS sorter.

For $CD34^+Lin^-$ purification, the $CD34^-$ fraction was depleted from cells expressing lineage antigens using a negative selection column (StemCell Technologies, Vancouver, BC, Canada).

Ex-vivo expansion of $CD34^{+/-}$ cell populations:

$CD34^+$ expressing purified cells above were cultured in 24-well Costar Cell Culture Clusters (Corning Inc., Corning, NY) or culture bags (American Fluoroseal Corp), at a concentration of 10^4 cells/ml in alpha medium (Biological Industries, Beit Haemek, Israel) supplemented with 10 % fetal bovine serum (FBS, Biological Industries). The following human recombinant cytokines were added: Thrombopoietin (TPO), interleukin-6 (IL-6), FLT-3 ligand and stem cell factor (SCF), all at final concentrations of 50 ng/ml each, though occasionally IL-3, at a concentration of 20 ng/ml, was added either together or instead of SCF. All cytokines used were purchased from Perpo Tech, Inc. (Rocky Hill, NJ). The cultures were incubated at 37 °C, 5 % CO_2, in a humidified atmosphere.

Alternatively, whole mononuclear fraction cells (MNC) were isolated, cultured and supplemented with cytokines, as above.

At weekly intervals, cell cultures were toped and semi-depopulated and were supplemented with fresh medium, serum and cytokines or supplemented with fresh growth medium, alone. At predetermined time points, cells were harvested, stained with trypan blue, counted, and cell morphology was determined via the use of cytospin (Shandon, UK)-prepared smears stained with May-Grunwald/Giemsa solutions.

Nicotinamide was added to cell cultures at concentrations of 1, 5 or 10 mM for up to five weeks culture period.

Surface antigen analysis:

Cells were harvested, washed with a PBS solution containing 1 % bovine sera albumin (BSA) and 0.1 % sodium azide (Sigma), and stained at 4 °C for 60 minutes with fluorescein isothiocyanate or phycoerythrin-conjugated antibodies (all from

Immunoquality Products, the Netherlands). The cells were then washed with the same buffer and analyzed by FACS caliber or Facstarplus flow cytometers. Cells were passed at a rate of 1000 cells/second, using saline as the sheath fluid. A 488 nm argon laser beam served as the light source for excitation. Emission of ten thousand cells was measured using logarithmic amplification, and analyzed using CellQuest software. Negative control staining of cells was accomplished with mouse IgG-PE (Dako A/S Glostrup, Denmark) and mouse IgG-FITC (BD Biosciences, Erembodegem, Belgium).

Determination of CD34 and other hematopoietic marker expression:

CD34 surface expression on short and long-term cultures initiated either with purified $CD34^+$ cells or the entire MNC fraction was determined as follows: $CD34^+$ cells were positively reselected (Miltenyi kit) and counted. Purity was confirmed by subsequent FACS and cell morphology analysis.

Reselected $CD34^+$ cell subsets were stained for the following combination of antigens: CD34PE/CD38FITC and CD34PE/38, 33, 14, 15, 3, 4, 61, 19 (Lin) FITC. The fraction positive for CD34 and negative for CD38 was defined as $CD34^+CD38^-$. The fraction positive for CD34 and negative for LIN was defined as $CD34^+Lin^-$ cell fraction.

Cell population calculations:

FACS analysis results are given as percentage values of cells. Absolute numbers of subsets are calculated from the absolute number of $CD34^+$ cells.

Determination of baseline levels of $CD34^+$/CD38- and $CD34^+/Lin^-$ cells was conducted as follows: $CD34^+$ cells were purified from 3 thawed cord blood units and stained for the above markers. The mean of these experiments was considered as the baseline value.

Total cell counts, numbers of $CD34^+$ cells and subsets are presented as cumulative numbers, with the assumption that the cultures had not been passaged; i.e., the number of cells per ml were multiplied by the number of passages performed.

Results

Table 1 illustrates the expansion of the CD34+ hematopoietic cells in nicotinamide, assessed at three and five weeks. Table 2 shows the effect of culturing hematopoietic cells with nicotinamide on expansion of CD34+/CD38- and CD34+/Lin- subsets after three and five weeks.

Table 1-Expansion of CD34+ Cells

	Total Cells		CD34+ Cells	
Treatment	X 10^4 (cumulative)	Fold Expansion	X 10^4 (cumulative)	Fold Expansion
Control (cytokines)	20160	1680	708	59
Nicotinamide 5mM 3 weeks	18240	1520	1061	88
Nicotinamide 5mM 5 weeks	24768	2067	1152	96

Table 2-Expansion of CD34+ Subsets

	CD34+/CD38- Cells		
Treatment	%CD34+/CD38-	X10^4(cumulative)	Fold Expansion
Control (cytokines)	0.48	3	3
Nicotinamide 5mM 3 weeks	7.84	83	47
Nicotinamide 5mM 5 weeks	29.87	344	178
	CD34+/Lin- Cells		
Treatment	%CD34+/Lin-	X10^4(cumulative)	Fold Expansion
Control (cytokines)	0.23	1.63	27
Nicotinamide 5mM 3 weeks	3.94	41.80	683
Nicotinamide 5mM 5 weeks	25.77	296.87	4851

Note the enhanced expansion of CD34+ cells over 5 weeks, and the exponential increase in the early progenitor CD34+/CD38- and CD34+/Lin- subsets after 5 weeks, as compared to 3 weeks exposure to nicotinamide.

As a person signing below, I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the Application or any patent issued thereon.

26691

October 27, 2007

Dr. Tony Peled

Enc.: CV of Dr. Tony Peled

CURRICULUM VITAE of Dr. Tony Peled

Academic education

Ph.D 1987-1995 Hebrew University- Hadassah Med. School, Jerusalem, Israel
Research subject: Purification of a serum derived factor (SDF) inducing differentiation of leukemic cells
M.Sc 1984-1987 Hebrew University- Hadassah Med. School, Jerusalem, Israel
Research subject: cellular pathways regulating differentiation of leukemic cells
B.Sc 1980-1983 The Hebrew University of Jerusalem

Professional experience

1998- 2000, Founder and the Chief Scientist of the stem cell startup company, Gamida-cell Ltd., Jerusalem, Israel: During this period I was in charge of the establishment of new labs including equipment, analytical methods and technicians training as well as continuation of the research described above and evaluation of the possibility to translate the results into a cell product.

2000- 2002, Head of research and development (R&D) Gamida-cell Ltd., Jerusalem, Israel: I was in charge of the development of a cell product for cord blood transplantation indications including preparing it for the submission to the USA, Food and Drug Administration (FDA), and escorting it all along the procedure vis a vis the FDA.

2002-2004, Head of new technologies, Gamida-cell Ltd. Jerusalem, Israel: In this position I developed novel technologies and evaluating the possible applications of these technologies for additional indications in the stem-cell therapy arena.

2005-2007, Chief scientist and vice president of Gamida-cell Ltd. Jerusalem, Israel

List of Publications

- **Nicotinamide, a form of vitamin B3, promotes expansion, homing and engraftment of cultured CD34+ cells**
  Peled T, Adi S, Goudsmid N, Daniely Y, Nagler A, Fibach E
  Submitted

- **Labile copper pool modulates hematopoietic progenitor cell self-renewal: Effect of a stable TEPA-copper complex**
  Peled T, Glukhman E , Hasson N, Adi S , Assor H, Yudin D, Landor C, Mandel J, Landau E, Nagler A and Fibach E
  Submitted to the Experimental Hematology journal 2005

- **Epigenetic modulation of commitment and differentiation of hematopoietic progenitor cells by low-molecular-weight linear polyamine copper-chelators**
  Peled T, Daniely Y
  Experimental Hematology: Editorial paper to be published in 2005

- **Pre-clinical development of cord blood-derived progenitor cell graft expanded ex vivo with cytokines and the polyamine copper chelator tetraethylenepentamine**
  T Peled, J Mandel, Rn Goudsmid, C Landor, N Hasson, D Harati, M Austin, A Hasson, E Fibach, Ej Shpall, A Nagler

Cytotherapy 2004 Volume 6, Number 4: 344-355

- **Transplantation of ex vivo expanded cord blood**
  Shpall Ej E, McNiece Ik I, De Lima M, McMannis Jd J, Robinson S, Peled T, Champlin Re R
  Biol Blood Marrow Transplant.2004 Oct;10(10):738.

- **Linear polyamine copper chelator tetraethylenepentamine augments long-term ex vivo expansion of cord blood-derived CD34+ cells and increases their engraftment potential in NOD/SCID mice**
  Peled T, Landau E, Mandel J, et all
  Exp Hematol. 2004 Jun;32(6):547-55.

- **The effect of tetraethylenepentamine, a synthetic copper chelating polyamine, on expression of CD34 and CD38 antigens on normal and leukemic hematopoietic cells**
  Prus E, Peled T, Fibach E
  Leuk Lymphoma 2004 Mar;45(3):583-9.

- **Cellular copper content modulates differentiation and self-renewal in cultures of cord blood-derived CD34+ cells**
  Peled T, Landau E, Prus E, Treves AJ, Nagler A, Fibach E
  Br J Haematol. 2002 Mar;116(3):655-61

- **The Effect of Human Myelomonocytic Leukemic Cell Line (M20) Derived IL-1 Inhibitor on Human Erythoric Cell Development**
  Fibach E, Rigel M, Peled T, Treves AJ, Barak V
  Leuk Lymphoma 1994 Oct;15(3-4)327-32

- **Effect of M20 Interleukin-1 Inhibitor on Normal and Leukemic Human Myeloid Progenitors**
  Peled T, Rigel M, Peritt D, Fibach E, Treves AJ, Barak V
  Blood 1992 Mar 1;79(5):1172-7

- **Isolation and Characterization of HL-60 Cell Variants with Different Potentials for Spontaneous Differentiation**
  Fibach E, Peled T, Fibich T, Rachmilewitz EA
  Leukemia 1991 Oct;5(10):912-6

- **Self-Renewal and Commitment to Differentiation of Human Leukemic Promyelocytic Cells (HL-60)**
  Fibach E, Peled T, Rachmilewitz EA
  J Cell Physiol. 1982 Oct;113(1):152-8

- **Changes in Cell Kinetics Associated with Differentiation of a Human Promyelocytic Cell Line (HL-60)**
  Fibach E, Peled T, Rachmilewitz EA
  Cell Tissue Kinet. 1982 Jul;15(4):423-9

- **Modulation of the Maturation of Human Leukemic Promyelocytes (HL-60) to Granulocytes or Macrophages**

Fibach E, Peled T, Treves A, Kornberg A, Rachmilewitz EA
Leuk Res. 1982 ;6(6):781-90

International scientific meetings
(published abstracts, poster and oral presentations)

- **Nicotinamide, a Potent SIRT2 Inhibitor, Delays Differentiation of Hematopoietic Progenitor Cells.**
  Peled T, Adi S, Glukhman E, Grynspan F, Nagler A, Fibach E, Daniely Y.
  ASH 2004 Publication
  Blood 2004, Volume 104, Issue 11

- **Cell Cycle Progression and Self-Renewal Divisions of Cord Blood Derived CD34+ Cells Treated with the Polyamine Copper-Chelator Tetraethylenepentamine.**
  Peled T, Rosenheimer-Goudsmid N, Grynspan F, Adi S, Landau E, Nagler A, Fibach E, Daniely Y.
  ASH 2004 Publication
  Blood 2004, Volume 104, Issue 11

- **Cord Blood Derived CD34+ and AC133+ Progenitor Cells Ex-Vivo Expanded in the Presence of Tetraethylenepentamine: Reproducibility among Cord Blood Units.**
  Grynspan F, Peled T, Rosenheimer-Goudsmid N, Lador H, Hasson N, Mandel J, Landau E, Glukhman E, Assaf H, Yudin D, Adi S, Olesinski E, Daniely Y, Hasson A, Harati D, Nagler A, Fibach E, Shpall E.
  ASH 2004 **Oral presentation**
  Blood 2004, Volume 104, Issue 11

- **Ex-Vivo Expanded Human Bone Marrow-Derived AC133+ Cells To Treat Myocardial Infarction.**
  Grynspan F, Marikovsky M, Landau E, Peled T, Nagler A, Holbova R, Guetta V, Fuchs S, Feinberg S, Leor J.
  ASH 2004 **Oral presentation**
  Blood 2004, Volume 104, Issue 11

- **Transplantation of Cord Blood Expanded Ex Vivo with Copper Chelator.**
  Shpall E, de Lima M, Chan K, Champlin R, Gee A, Thall P, Komanduri K, Couriel D, Andersson B, Hosing C, Giralt S, Safa Karandish S, Sadeghi T, Muriera B, Peled T, Grynspan F, Nagler A, McMannis J.
  ASH 2004 **Poster presentation**
  Blood 2004, Volume 104, Issue 11

- **Expansion of Human Umbilical Cord Blood-Derived $CD34^+$ Stem/Progenitor Cells to treat Myocardial Infarction.**
  Nagler A, Grynspan F, Peled T, Mandel J, Guetta, E, Holbova R, Feinberg MS, Daniely Y, Leor J.
  ISEH 2004 **Poster presentation**

- **Cell cycle progression and self-renewal divisions of CB derived CD34+ cells treated with the polyamine copper-chelator TEPA**
  Peled T, Goudsmid R N, Adi S, Landau E, Nagler A, Fibach E, Daniely Y.
  ISEH 2004 **Poster presentation**

- **Ex-vivo Expansion of Human Bone Marrow-Derived AC133+ Cells in the Presence of a Polyamine Copper Chelator.**
  Landau E, Marikovsky M, Yudin D, Nagler A, Peled T, Hasson A, Bulvik S, Grynspan F.
  ISCT 2004 **Poster presentation**

- **"Linear Polyamines Support Self-Renewing Division of Hematopoietic Early Progenitor Cells: Mechanism Of Activity".**
  Peled T, Adi S, Glukhman E, Hasson N, Assor H, Grynspan F, Nagler A, Fibach E.
  ISCT 2004 **Poster presentation**

- **TEPA Augments the Ex-Vivo and In-Vivo Potential of Cord Blood Derived CD34+ Cells: From Basic Science to Clinical Trials.**
  Peled T, Rubinstein P, Kurtzberg J, Nagler A, Fibach E, Shpall EJ.
  ASH 2003 **Poster presentation**
  Blood 2003 Volume 102, Issue 11

- **Expansion of Human Umbilical Cord Blood-Derived CD34+ Stem/Progenitor Cells To Treat Myocardial Infarction.**
  Nagler A, Grynspan F, Peled T, Mandel J, Guetta E, Holbova R, Feinberg MS, Leor J.
  ASH 2003 **Poster presentation**
  Blood 2003 Volume 102, Issue 11

- **The Copper Chelator Tetraethylenepentamine Augments Long-Term Ex-Vivo Expansion of Hepatic Progenitor Cells.**
  Peled T, Lador H, Grynspan F, Fibach E, Rosenheimer N.
  ASH 2003 Publication
  Blood 2003 Volume 102, Issue 11

- **The linear polyamine copper chelator Tetraethylenpolyamine (TEPA) Augments Extended Long-Term Ex Vivo Expansion of CD34+ Cells and Increase Their Potential to Engraft NOD/SCID Mice.**
  Peled T, Mandel J, Landau E, Nagler A, Shpall EJ, Fibach E.
  ISCT 2003 **Oral presentation**

- **Polyamine Copper Chelators Augment the Ex vivo and In vivo Potential of Cultured Hematopoietic CD34+ Cells: Mechanism of Activity**
  Peled T, Glukhman E, Hasson N, Adi S, Lador H, Ludin D, Fibach E.
  ISEH 2003 **Poster presentation**

- **Pre-clinical Development of Ex Vivo Expanded CB Derived Progenitor Cell Graft with Cytokines and the Polyamine Copper Chelator TEPA**
  Peled T, Rubinstein P, Kurtzberg J, Nagler A, Fibach E, Shpall EJ.
  ISEH 2003 **Oral presentation**

- **Copper Chelators Sustain Long-Term Expansion of Cord-Blood CD_{34}^{+} Cultures Intiated with IL-3 and G-CSF - Late-Acting, Differentiation-Inducing Cytokines**
  Peled T, Treves AJ, Nagler A, Alachalal U, Levy M, Fibach E
  ASH 2000 **Poster presentation**
  Blood 2000 96:773a Abstract No. 3343

- **Regulation of Long-Term Expansion of Hemopoietic Stem/Progenitor Cells (HPC) by Intracellular Copper Content**

Peled T, Landau E, Prus E, Treves AJ, Nagler A, Alchalal Levi M, Fibach E
ASH 2000 **Oral presentation**
Blood 2000 96:776a Abstract No. 3359

- **Long-Term Expansion of Hematopoietic Progenitors (HPC) from Human Umbilical Cord blood by Copper Chelators**
  Peled T, Treves AJ, Nagler A, Elchalal U, Rachmilewitz EA, Fibach E
  ASH 1999 **Oral presentation**
  Blood 1999 Dec;Abst.3125

- **Involvment of Copper in Proliferation and Differentiation of Hematopoietic Progenitors**
  Peled T, Treves AJ, Fibach E
  ASH 1999 **Poster presentation**
  Blood. 1999 Dec;Abst.3755

- **Identification of a Serum Derived Differentiation Inducing Activity as the Copper Binding Protein Ceruloplasmin**
  Fibach E, Peled T, Treves A, Rachmilewitz EA
  ASH 1998 **Poster presentation**
  Blood 1998 Dec; No. 2551

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